inhibitors. DNA content was monitored by flow cytometry and the levels of mitotic regulators were measured by immunoblotting.
Results: SU6656 and geraldol induced the degradation of the spindle assembly checkpoint protein BubR1, the anaphase promoting complex activator Cdc20, and the CDK1 regulatory subunit cyclin B1. This degradation was not observed during completion of mitosis or early G1 in T98G cells. The effects of SU6656 and geraldol were observed in cells arrested at mitosis but not in cycling cells. Simultaneous exposure of cells to a proteasome inhibitor or a caspase inhibitor and SU6656 or geraldol prevented protein degradation and mitotic slippage. Further, MCF-7 cells, which are deficient in caspase-3, were resistant to induction of mitotic slippage by SU6656 or geraldol. Stable transfection of MCF-7 cells was used to examine the requirement for caspase-3 activity for mitotic slippage.
Conclusions: The mitotic slippage inducers SU6656 and geraldol act via proteasome- and caspase-dependent degradation of proteins required to maintain mitotic arrest.

732 Role of microRNAs and microRNA machinery in the pathogenesis of diffuse large B-cell lymphomas

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Diffuse large B-cell lymphoma (DLBCL) is one of the most common cancers among all B-cell non-Hodgkins lymphomas and is characterized by high genetical, morphological and clinical heterogeneity. Although different studies have been conducted to investigate the dysregulation of microRNAs in DLBCLs, the role of these small non-coding RNAs is still not well understood. In this study we aimed to explore the contribution of microRNA expression alteration in DLBCL carcinogenesis. Using miRNA microarray and qRT-PCR approaches, we analyzed global microRNA expression and their processing machinery in a set of 70 DLBCLs (62 *de novo* and 8 transformed) and 10 non-neoplastic lymph nodes (LN). Our results show a significant over-expression of *TRBP* gene in tumours as compared to LN suggesting its potential role in development/progression of diffuse large B-cell lymphoma. Further, a comparison between *de novo* and transformed cases revealed an up-regulation of *DROSHA*, *TRBP* and *PACT* in *de novo* DLBCLs. Deregulated microRNAs, identified by microarray analysis, were evaluated in relation to clinical and molecular characteristics of lymphoma cases. Forty-four differentially expressed microRNAs could distinctly classified DLBCL tumours from normal lymph node samples. We also identified 11 candidate microRNAs that could distinguish GCB-DLBCL subtype from non-GCB. Subsets of down-regulated microRNAs were associated with high expression of *BCL6* and *IRF4*, and higher expression of *miR-494* and *miR-638* was observed in advanced stages of DLBCLs. Furthermore we found an association between microRNA deregulation and high expression of *DROSHA*, *DICER* and *TRBP* in lymphoma tumours.
In conclusion, our findings give new insights in the understanding the role microRNA machinery and microRNAs in the diffuse large B-cell lymphomas carcinogenesis.

733 HER2 status in breast carcinomas: comparison between silver in situ hybridization, chromogenic in situ hybridization and fluorescence in situ hybridization

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Introduction: Determination of HER2 amplification in breast carcinoma was regularly reported using fluorescence in situ hybridization (FISH) as a golden method. However, HER2 FISH method required specialized fluorescence microscope, high cost and their signals definitely faded over time causing it to be impractical for routine laboratories. Chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH) have been reported to be suitable option to FISH as their stability of signals were archival. The aim of this study was to evaluate HER2 and compare it using different techniques, SISH, CISH and FISH.
Methods and Materials: HER2 expression was evaluated by immunohistochemistry (IHC) in 234 breast carcinoma samples. Whole sections of HER2 of 26 cases with borderline positive (2+) IHC were further validated by using manual dual-colour FISH, manual single colour CISH and automated single colour SISH.
Results: By IHC, the samples for HER2 were negative in 70.1%, 1+ in 6.8%, 2+ in 12.2% and 3+ in 10.9%. The 26 cases that were borderline positive (2+) IHC were further analysed for FISH and 88.0% (22) of the cases showed HER2 amplification, 12.0% showed no amplification and 2 cases were non-interpretable. By using CISH, 22 cases showed low to high amplification with more than 5 dots to clusters in more than >50% of carcinoma cells and one case was equivocal. Whereas by using SISH, 19 cases were HER2 amplified and 5 cases were not assessable due to absence of adequate SISH signals. A high level of concordance between FISH and CISH, FISH and SISH, CISH and SISH were observed in 92% ($p = 0.029$, $k = 0.621$), 95% ($p = 0.095$, $k = 0.644$) and 100% ($p = 0.05$, $k = 1.00$) respectively. Technically, we experienced that SISH saved time as it could be done in a short time compared to CISH and FISH. Besides, the signals when using SISH were seen under the ordinary light microscope showed discrete metallic silver black and have a permanent result compared to CISH whose signals became less discrete over long period. FISH method was time consuming and laborious. However, CISH equipment was less expensive than FISH or SISH.
Conclusion: This study shows that CISH and SISH are practical methods that can detect HER2 amplification and may be an alternative used in routine laboratories which are not equipped to do FISH.

734 Identification of gene expression alterations associated with fibrosis in breast cancer survivors

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Advances in detection and treatment of breast cancer have lead to an increasing number of cancer survivors. In consequence, late effects and optimal quality of life have become new important end points in cancer care. Radiotherapy (RT) is an established treatment for breast cancer, however irradiation of normal tissue can induce side effects. Fibrosis is a frequent late side effect among breast cancer survivors treated with RT. Considered to be a consequence of complex biological processes, fibrosis gradually develops over several years. Although fibrosis has been extensively studied, high throughput assays have opened new research avenues which can be used as target discovery tools to develop clinically useful late-effects signatures.
This study included 253 survivors derived from a cohort of survivors, treated for breast cancer stage II/III with adjuvant RT at the Norwegian Radium Hospital between 1998 and 2002. The women were in 2004 invited to participate in a study assessing late side effects. A clinical examination evaluating fibrosis in the breast, axilla and supraclavicular area was performed and blood samples for RNA analyses were drawn in PAX tubes. Gene expression analysis was conducted using Illumina Human-6 version 2 expression beadchips and the data were analyzed using R version 2.8.0 with tools from the Bioconductor project.
Our analyses showed a correlation between age and fibrosis and chemotherapy and fibrosis, thus all the analyses were adjusted for these confounders. Of note, the occurrence of telangiectasia significantly correlated with fibrosis, suggesting that the patients experiencing both effects might constitute a subgroup of survivors, ultra-sensitive to radiation. Preliminary analyses show fibrosis to be significantly associated with global gene expression (p-value = 0.03). Multivariate gene-wise linear analysis identified 87 genes significantly associated with fibrosis (fdr < 0.15). Functional annotation enrichment analyses reveal that these genes are involved in cellular processes, gene expression, cellular component organization and biogenesis, intracellular transport, establishment of cellular localization and chromatin modification.
Breast cancer survivors with fibrosis show a different blood gene expression compared to non-fibrotic survivors. Finally, by analyzing our dataset using gene lists from several related studies in the literature, we aim to identify relevant biological pathways involved in fibrosis.

735 PTEN gene promoter methylation in endometrial and ovarian tumours

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Background: Tumour suppressor gene *PTEN* is implicated in the pathogenesis of several familial and sporadic cancers. The aim of our study was to analyze the possible existence of alterations in *PTEN* gene promoter in endometrial and ovarian tumours.
Material and Methods: The study included 34 patients with endometrial cancer, 26 – with endometrial hyperplasia, 24 – with ovarian cancer and 17 – with benign ovarian tumours. Normal endometrium tissue samples were obtained from 20 healthy women during therapeutic abortion. Genomic DNA was isolated and the −1148 to −727 promoter region called *PN-1* was amplified by PCR. The genomic DNA was treated with sodium bisulfite and used as a template for the amplification of the 618 bp fragment (*PN-2*) including the

PN-1 locus and flanking sequences (−1214 to −596). The PCR products were sequenced.
Results: We found that efficacy of PCR amplification of *PN-1* in tumour DNA samples depended on the presence of PCR enhancers 2-pirrolidone and/or dimethylsulfoxide (DMSO) in the reaction mixture. Three classes of DNA templates were isolated from different tumours. The first group marked P+D+ included 60 tumour DNA samples in which the *PN-1* was amplified only in the presence both 2-pyrrolidone and DMSO. The second group (P-D+) included 27 samples in which this locus was not amplified in the presence of 2-pyrrolidone, but the PCR product was generated when DMSO instead of 2-pyrrolidone was added. Neither 2-pyrrolidone nor DMSO could stimulate PCR amplification of the *PN-1* in the 14 DNA samples (P−D−) belonging to the third group. We supposed that DNA methylation might influence the efficacy of the *PN-1* PCR amplification. To prove our hypothesis methylation status of *PN-2* region was examined. In five tumour DNA belonging to P+D+ group and in five DNA samples from normal endometrium no methylation of *PTEN* promoter cytosine residues was detected. The five tumour DNA of P−D+ group revealed 51−79% of methylated CpG dinucleotides. In this case the non-CpG methylation was also observed. In DNA sample belonging to the P−D− group the fraction of CpG methylation in *PN-2* was 67.4%. In all cases the methylated cytosines were localized in the range of −1036 to −618. In addition to CpG the methylation at CpA, CpC and CpT sites was observed.
Conclusion: We revealed aberrant *PTEN* promoter methylation (epimutations) in endometrial and ovarian tumours and showed that methylation affects PCR amplification of *PTEN* promoter region.

736 The expression of WWOX tumour suppressor gene in colorectal cancer

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Background: *WWOX* gene is located in chromosome region 16q23.3–24.1 (common fragile site FRA16D), an area which is affected by frequent allelic losses in breast cancer. The evidence for tumour suppressor activity was first demonstrated in several cancer cell lines, however numerous studies showed either loss or reduction of the *WWOX* expression in a variety of tumours, including breast and lung cancer. The most common way of affecting *WWOX* function in cancer cells occurs through hemizygous deletions, while point mutations are rare. A number of studies showed that in some cancer types hypermethylation of *WWOX* promoter could be of some importance. Recently, a set of complex heterozygous deletions manifesting as homozygous loss was found at FRA16D in the HCT116 colon cancer cell line. Although this resulted in removing exons 6 to 8 of one *WWOX* transcript, it did not prevent the transcription of wild-type *WWOX* from 3rd allele. In order to clarify the role of *WWOX* gene in colorectal cancer (CRC) tissues, we analysed 99 tumour samples and 4 CRC cell lines: HT-29, HCT116, SW480 and SW620.
Material and Methods: The study was approved by the local Ethics Committee. Informed consent was obtained from all patients. Tissue samples were stored at −80°C until RNA extraction. All qPCR reactions were performed in duplicate, with EvaGreen dye and Corbett Research RG-3000 platform. LOH status was determined by three STS markers: D16S3096, D16S504, D16S518. Methylation status of two *WWOX* promoter regions was performed by *MethylScreen* method utilising qPCR assay on templates generated by combined restriction digestions.
Results: Relative *WWOX* expression in CRC tissues ranged from 0 to 123.18 (median 7.66). In the studied population we did not find any significant hemizygosity suggesting LOH at the studied loci. Also, there was no significant methylation of two examined regions. We found significant difference of DFS in patients with relatively high and low *WWOX* expression ($HR = 0.39$; $p = 0.0452$), but in multivariate analysis it was not an independent prognostic factor. *WWOX* expression correlated with expression of BCL2($r = 0.3996$; $p = 0.0001$), BAX ($r = -0.2671$; $p = 0.0082$) and CCNE1 ($r = -0.3579$; $p = 0.0005$).
Conclusions: Our data suggest that, unlike other tumours, *WWOX* expression in CRC is affected by different mechanisms than deletion or methylation. *WWOX* expression in CRC tumours correlated with expression of genes responsible for cell cycle regulation.
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737 The role of WWOX tumour suppressor gene in colorectal cancerogenesis − a microarray study on HT29 colon cancer cell line

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Background: Colorectal cancer is one of the leading cause of cancer-related deaths in both men and women in western countries. Nowadays, there are three recognized distinct molecular pathways of colon cell cancer transformation. The most common way is an acquisition of chromosomal instability (CIN). Microsatelite instability phenotype(MSI) and CpG Island Methylator Phenotype(CIMP) constitute for the other two pathways. Each of this cancerogenesis phenotype is characterized by molecular profile of genomic, transcryptomic and proteomic alterations.
WWOX is a tumour suppressor gene that spans the common fragile site FRA16D. It has been proven that *WWOX* participates in controlling expression of genes which are responsible for tissue morphogenesis and cell differentiation. Its altered expression has been demonstrated in many tumour types. Moreover, reduction of *WWOX* expression correlates with more aggressive disease stage and higher mortality rate (breast, gastric, lung cancer).
Materials and Methods: Experiments were performed on HT29 colon cancer cell line transfected with *WWOX* cDNA.
Using real-time RT PCR we estimated relevant expression level of 8 cancer marker genes(apoptosis, proliferation, adhesion and cell cycle regulation genes).
We employed whole genome, oligonucleotide microarrays(Human OneArray™;Phalanx Biotech) to assess the influence of *WWOX* on gene expression profiles. Moreover, we performed biological test of anchorage independent growth.
Results: Analysis of microarrays evaluated over 300 differentialy expressed genes in result of increased *WWOX* expression ($p < 0.05$). Our study demonstrated that *WWOX* inhibits expression of genes that are involved in cell cycle progression, WNT and Cadherin signaling pathways and cytoskeletal regulation by Rho GTPase. Genes related to apoptosis and FAS signaling pathway are upregulated. Microarray results are consistent with real time RT-PCR and will be confirmed with Western-Blott and RT-PCR for chosen genes.
Moreover, there was complete inhibition of cell growth in soft agar in cell culture with higher expression of *WWOX* gene.
Conclusions: Microarray gene expression study confirmed the role of *WWOX* in regulation of important pathways in cancerogenesis. As we assumed it has major impact on apoptosis, cell cycle regulation and WNT pathway inhibition in HT29 colon cancer cells.

738 WWOX tumour suppressor gene is affected in glioblastoma multiforme

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Background: Glioblastoma multiforme (GBM) is the most common type of primary brain tumour in adults. This neoplasm is highly lethal with an average survival about 1 year. *WWOX*, a tumour suppressor gene located in a common fragile site FRA16D, is involved in carcinogenesis and cancer progression in many different cancers. Reduced *WWOX* expression is associated with more aggressive phenotype and poor patient outcome in several cancers. Our aim was to investigate *WWOX* expression alternations and its correlations with proliferation, apoptosis and signal trafficking in GBM. We evaluated methylation level of *WWOX* promoter and percentage of loss of heterozygosity (LOH) in *WWOX* genomic region. We also analysed the correlation between mRNA level of *WWOX* and other cancer related genes such as *Ki67*, *Bcl2*, *Bax*, *EGFR*, *ErbB4* (splice variants: *JM-a* and *JM-b*).
Material and Methods: Using real-time RT-PCR we analysed expression levels of 7 genes in 59 cases of GBM. LOH was assessed in 63 patients by high resolution melting. Allelic losses were analyzed for three microsatellite markers: D16S504, D16S518, D16S3096. Methylation detection was performed for two regions of *WWOX* promoter with high contents of CpG. The examination was conducted by MethylScreen method in 67 patients.
Results: We observed a relatively high percentage of LOH for two out of three analysed microsatellites: 38.5% (D16S3096) and 54.5% (D16S504). Concurrent analysis of *WWOX* expression level in reference to promoter methylation and microsatellite markers state revealed a difference in *WWOX* expression in homo and heterozygotes. The highest expression was exhibited by unmethylated, heterozygous samples while the lowest by methylated, homozygous. Loss of heterozygosity lowered expression level in unmethylated samples (with exception of D15S504). Promoter methylation considerably